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NONPOLAR LIPID METHYLATION—IDENTIFICATION OF NONPOLAR METHYLATED PRODUCTS SYNTHESIZED BY RAT BASOPHILIC LEUKEMIA CELLS, RETINA AND PAROTID

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Abstract—Incorporation of radioactivity from [^{3}H- or ^{14}C-methyl]methionine into nonpolar lipids has been investigated in rat basophilic leukemia (RBL) cells, retina, and rat parotid gland. These nonpolar methylated lipids were extracted into heptane and characterized by thin-layer chromatography, high performance liquid chromatography, gas chromatography, and mass spectrometry. The major methylated nonpolar lipid product in the RBL cells themselves was ubiquinone-9, which accounted for about 90% of the nonpolar lipid and 20–30% of the total radioactive lipid formed. There was a modest increase in the methylation of nonpolar lipids upon stimulation of the RBL cells with IgE and anti-IgE, but the significance of this change is uncertain. In contrast to whole cells, RBL membrane fractions (incubated with [^{3}H-methyl]-*S*-adenosylmethionine) incorporated radioactivity primarily into fatty acid methyl esters and not ubiquinone. A third product, 2-(methylthio)-benzothiazole, was formed by RBL cells, retina and minced parotid upon incubation in enriched media. This product was formed enzymatically, apparently by the known enzyme *S*-thiolmethyltransferase, using the thiol substrate which contaminates these media. Evidence suggests that the enzyme may reside, at least in part, on the surface of the cells.

Methionine, acting through *S*-adenosylmethionine (SAM), is the methyl donor for numerous transmethylation reactions [1]. Recently, there has been increased interest in the methylation of lipids and, in particular, of phospholipids [2, 3]. In addition to these polar lipids, however, methylated nonpolar lipids are formed by tissues and homogenates. We undertook to identify these nonpolar methylated products in several systems, using lung membranes [4], rat parotid, retina and rat basophilic leukemia cells. Lung membrane fractions enzymatically formed fatty acid methyl esters as their major lipid product [4].

Here we report that ubiquinone-9 (Q_9) and to a lesser extent fatty acid methyl esters are the nonpolar methylated lipid products formed by rat basophilic leukemia cells. Homogenates of these cells formed fatty acid methyl esters and not ubiquinone-9. We also report the inadvertent biosynthesis of the xenobiotic 2-(methylthio)-benzothiazole due to the presence of the thiol substrate in enriched media (Eagle's MEM or RPMI 1640 as compared to buffer or salt solutions). Evidence suggests that the enzyme responsible, *S*-thiolmethyltransferase, may reside, at least in part, on the surface of cells.

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MATERIALS AND METHODS

Materials. Radioisotopes were purchased from the New England Nuclear Corp. (Boston, MA). Fatty acid methyl esters, ubiquinone-10, and homocysteine thiolactone were purchased from the Sigma Chemical Co. (St. Louis, MO), 2-thiobenzothiazole from the Aldrich Chemical Co. (Milwaukee, WI), and 3-deazaadenosine from Southern Research Institute (Birmingham, AL). Eagle's minimum essential medium (MEM) was purchased from M. A. Bioproducts (Walkersville, MD) and RPMI 1640 from the Grand Island Biological Co. (Grand Island, NY). 2-(Methylthio)-benzothiazole was prepared on a microgram scale from diazomethane and 2-thiobenzothiazole using standard conditions [5]. Rat monoclonal immunoglobulin E (IgE) [6] and anti-IgE antibodies [7] were prepared as described previously.

Cells. A secreting subline of the rat basophilic leukemia 2H3 cell line [8] was employed. The line was maintained as described previously [8]. Cells were reseeded weekly using 4×10^6 cells/150 cm^2 flasks (Costar) and were grown in 35 ml of carbonate-buffered minimum essential medium—Eagle's (MEM), fortified with 16% heat-inactivated fetal bovine serum (GIBCO), L-glutamine, and gentamicin.

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Cells were subcultured for 3–4 days under the same conditions, released by trypsin-versene, and quenched with 16% serum in MEM. Harvested cells were spun down (10 min × 800 rpm), washed twice in phosphate-buffered saline (PBS), pH 7.4, once in Tyrode's solution [NaCl, 135 mM; KCl 5 mM; $CaCl_2$, 1.8 mM; $MgCl_2$, 1 mM; glucose, 5.6 mM; gelatin, 0.05%; and 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES), 10 mM; pH 7.6], and suspended in Tyrode's solution (5×10^6 cells/ml).

Lipid methylation. Washed cells in Tyrode's solution ($3.0–5.0 \times 10^6$ cells/ml) (0.5 ml) were incubated with 12.5 μCi alcohol-free [^{3}H-methyl]-methionine (11–13 Ci/mmole) in test tubes at 37°. Rat retinas were incubated in 5.0 ml of RPMI 1640 medium minus methionine with 50 μCi of [^{3}H-methyl]methionine or 50 μCi of [^{3}H-methyl]-*S*-adenosylmethionine (12.9 Ci/mmole) for 60 min at 37°. Rat parotid glands were minced with a razor blade, and tissue from about one-fifth of a gland was incubated in 0.5 ml of RPMI 1640 medium minus methionine with 10 μCi of [^{3}H-methyl]methionine or 7.5 μCi [^{3}H-methyl]-*S*-adenosylmethionine. Reactions were stopped with 1 ml of ice-cold Tyrode's solution, the tubes were centrifuged (500 *g* × 10 min), and the medium was aspirated. When total lipid methylation was assayed, the pellets were extracted with chloroform–methanol (2 : 1) and the extract was washed three times with 0.1 M KCl–methanol (1 : 1). Measured aliquots of the chloroform extract were dried and counted; others were used for TLC. When nonpolar lipid methylation was assayed, the pellets were taken up in 83% ethanol and extracted with heptane. Aliquots of upper phase were counted directly or saved for further analysis. For long-term (2 hr or 24 hr) incubations and batch preparation, radioactive methionine was added to the flasks in which the cells were grown. At the end of the incubations, the medium was decanted, extracted with methylene chloride, and washed twice with saline. The cells were harvested, washed, and extracted with alcohol–heptane. For membrane methylation reactions, cells were homogenized in 50 mM Na phosphate buffer (pH 7.5) containing 5 mM $MgCl_2$. The 100,000 *g* pellet was then resuspended in the same buffer containing 0.1% Triton X-100 and incubated at 37° with [^{3}H-methyl]-*S*-adenosylmethionine (13 Ci/mmole).

Thin-layer chromatography (TLC) and high pressure liquid chromatography (HPLC) analysis. Samples or standards were spotted on silica gel G plates and developed in one of the following solvent systems: (1) chloroform–methanol–water (65 : 25 : 4); (2) benzene; (3) chloroform; or (4) heptane–diethyl ether–acetic acid (80 : 30 : 2). For HPLC, samples and/or standards were injected onto a 15 cm silica gel column (Supelco LC-Si) protected by a small guard column. The instrument was a Varian 8500. Samples were eluted with 50% methylene chloride in heptane at a rate of 1 ml/min, and 1-min fractions were collected.

Mass spectrometry. Electron impact mass spectra of HPLC purified methylated lipids were obtained with a Vacuum Generators 7070 mass spectrometer with a DS 2050 data system. For ubiquinone, samples were introduced via a solid probe inlet, and the instrument was operated at 3000 volts accelerating potential, 70 electron volts ionizing potential, and at an ion source temperature of 250°. Gas chromatography–mass spectrometry of volatile lipids was performed as previously described [4].

Stimulation of degranulation. Cells (5×10^6 cells/ml) were preincubated with rat IgE (0.7–1 μg/10^6 cells) for 1 hr at 35° and washed as described above. Degranulation was triggered by the addition of anti-IgE antibodies (1 μg/ml) to the cells in Tyrode's solution at 37°. [^{3}H]Serotonin release [9] was occasionally tested under these conditions. Induced release ranged between 35 and 50% per 30 min with spontaneous release of 9–13%. Cells grown in spinner culture showed little induced secretion (<6%).

RESULTS AND DISCUSSION

Nonpolar lipid methylation by RBL cells. Incubation of rat leukemic basophils with [^{3}H-methyl]-methionine resulted in the incorporation of radioactivity into several lipid-extractable products (Fig. 1, upper panel). After extraction with chloroform–methanol, about 50% of the recovered radioactivity migrated on TLC as polar lipids (presumably phospholipids), while about 20% migrated with the solvent front. There was also considerable radioactivity at the origin. After extraction with alcohol–heptane, more than 90% of the radioactivity

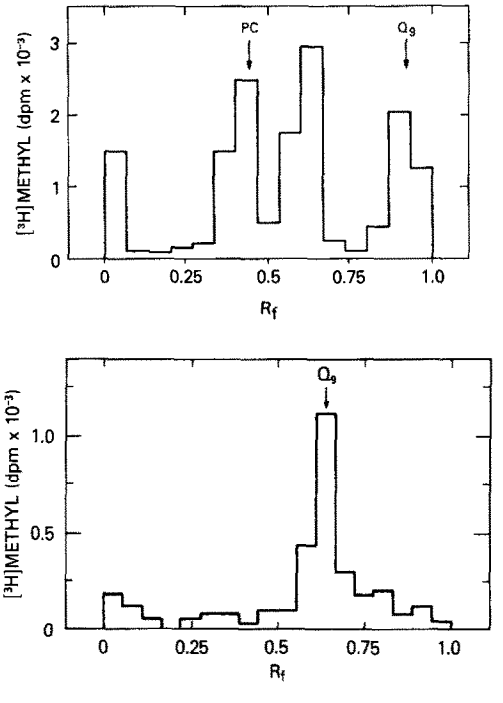

Fig. 1. TLC of methylated lipids. RBL cells (1.5×10^6 cells) were incubated with [^{3}H-methyl]methionine in Tyrode's solution for 30 min. Upper panel: Radioactive lipids were extracted with chloroform–methanol and the products were separated by TLC in chloroform–methanol–water (65 : 25 : 4). Lower panel: Radioactive lipids were extracted with alcohol–heptane and the products were separated on TLC in chloroform.

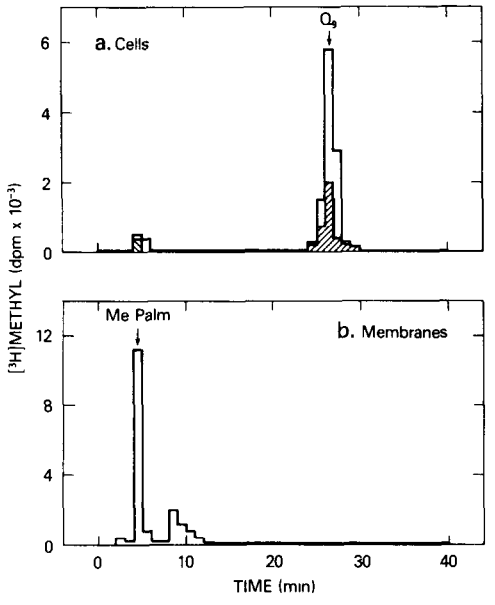

Fig. 2. HPLC of methylated nonpolar lipids. (a) Whole cells. RBL cells were incubated for 30 min in Tyrode's solution (hatched lines) or for 24 hr in Eagle's MEM with 25 μCi/ml [^{3}H-methyl]methionine. Nonpolar lipids were extracted with alcohol–heptane and analyzed by HPLC. Retention times of methyl palmitate (Me Palm) and Q_9 standards are indicated. (b) Membranes. RBL cell membranes (0.6 mg protein) were incubated with 10 μCi [^{3}H-methyl]-*S*-adenosylmethionine for 30 min in phosphate buffer (50 mM, pH 7.4) containing 5 mM $MgCl_2$ and 0.1% Triton X-100. Nonpolar lipids were extracted with alcohol–heptane and analyzed by HPLC.

migrated on TLC in nonpolar solvent as a single peak (Fig. 1, lower panel), which accounted for the radioactivity previously seen at the solvent front.

Methylation of the nonpolar product appeared to be enzymatic. It varied with time and the number of cells used and did not occur after incubation at 0°. Addition of 3-deazaadenosine and homocysteine thiolactone (which increases the levels of intracellular methyltransferase inhibitors [10]) markedly inhibited nonpolar lipid methylation, suggesting that *S*-adenosylmethionine was the actual methyl donor.

Identification of ubiquinone-9. The nonpolar lipid extracted into heptane from washed cells migrated like ubiquinone-9 on TLC in three different solvent systems. On HPLC, about 90% of the radioactivity eluted as a single peak (Fig. 2a). The same retention time was obtained whether cells were incubated for 30 min in Tyrode's solution or 24 hr in enriched medium. A small amount of radioactivity with the retention time of fatty acid methyl esters was also observed.

Identification of ubiquinone-9 was confirmed by mass spectrometry (Fig. 3). The spectrum shows particularly intense fragment ions at *m/z* 197 and 235. These are characteristic of ubiquinones and derive from the specific fragmentations of the isoprenoid chain indicated [11]. In addition to the intense ions at *m/z* 197 and 235, the lipid isolated from RBL cells exhibited molecular species at *m/z* 794 and 796, which are characteristic of ubiquinone-9 and its corresponding hydroquinone. This is the predominant ubiquinone in rat tissues [12]. Background ions between *m/z* 300 and 700 were also observed in other nonradioactive lipid fractions eluted from HPLC and were of unknown origin. The increased ratio of fragment ions 199/197 and 237/235 in the sample (as compared to unlabeled standard) reflected the incorporation of ^{14}C into the methoxy group of the ubiquinone. In the biosynthetic sequence of ubiquinones, methylation [13] to form the second methoxy group appears to be the last step [14–16].

Further evidence that the methyl group of [^{14}C-methyl]methionine was incorporated into the methoxy groups of ubiquinone was provided by the effect of ethanolic base on [^{14}C]-ubiquinone. The methoxy groups of ubiquinone have the unusual property of exchanging with methoxy or ethoxy groups in basic

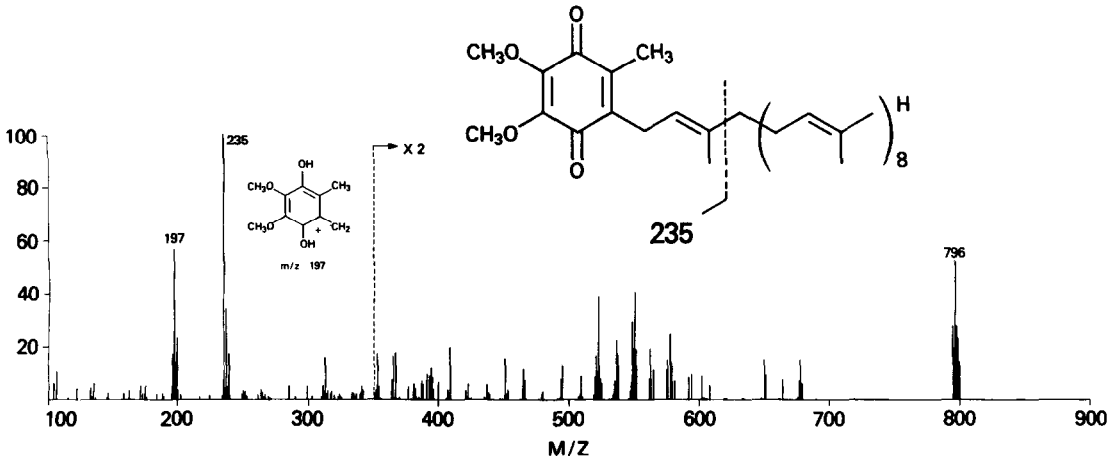

Fig. 3. Mass spectrum. RBL cells were grown for 24 hr in the presence of [^{14}C-methyl]methionine (59 mCi/mmole). Cells were harvested, washed, and extracted with alcohol–heptane. The major product was partially purified by HPLC and subjected to mass spectrometry. Dominant fragment ions at *m/z* 197 and 235 derive from the quinone nucleus as schematically depicted.

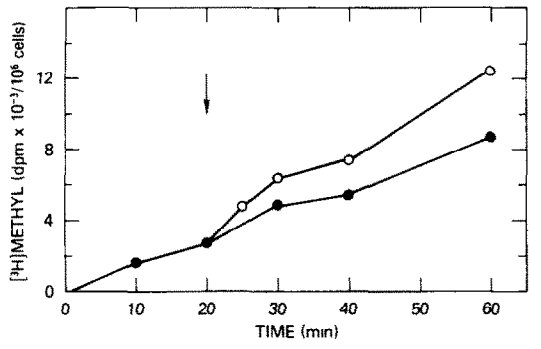

Fig. 4. Effect of stimulation on nonpolar lipid methylation. Cells were preincubated for 1 hr with IgE, washed, and then incubated in Tyrode's solution with [³H-methyl]methionine. After 20 min (↓) control cells received 12 μl of Tyrode's solution (●) and stimulated cells (○) received 12 μl of anti-IgE antibody. Nonpolar lipid methylation was assayed by alcohol–heptane extraction. Values shown are means of duplicate samples.

alcohol solutions [17]. This property permits the [³H- or ¹⁴C-methyl]ubiquinone to generate evaporable counts in base, as if it were a methyl ester. About 50% of the radioactivity in [³H-methyl]ubiquinone isolated from RBL cells became evaporable after incubation overnight at room temperature in ethanolic 0.1 N NaOH. No radioactivity was lost in the absence of base.

The subcellular distribution of ubiquinone-9, methylated after a 1-hr incubation of RBL cells with [³H]methionine, was examined by methods described previously [18]. The mitochondrial fraction had the most radioactivity, but significant incorporation was also seen in the microsomal membranes. Ubiquinones have been investigated extensively [16, 17, 19–23]. The mitochondrial localization and the participation of ubiquinones in electron transport are well known [24, 25]. The presence of ubiquinone in microsomal fractions has been shown previously by careful studies in rat liver and is not due to mitochondrial contamination [18]. The function of ubiquinone in non-mitochondrial membrane fractions has not been determined.

Effect of receptor stimulation. We tested the effect of receptor-mediated cell stimulation on nonpolar lipid methylation. Stimulation of RBL cells (which had been sensitized with IgE) by the addition of anti-IgE caused a small but significant increase (20–30%) in nonpolar lipid methylation (Fig. 4). Such increases were observed in eight of thirteen experiments of the kind shown. When grown in spinner culture these cells lose their capacity to release histamine upon stimulation with IgE and anti-IgE (unpublished observations). Such cultures showed the same basal levels of nonpolar lipid methylation but no change in response to stimulation (three experiments). Although ubiquinone-9 is the predominant product in the heptane extracts, the increase observed was too small to reliably identify the source of the increase under the experimental conditions used. Attempts to increase the effect (by using adherent cells or cells that had been grown in the absence of serum for 24 hr) were unsuccessful. Thus, the nature and mechanism of the effect of stimulation on nonpolar lipid methylation are uncertain.

Nonpolar lipid methylation in homogenates. When RBL cell membrane fractions were incubated with [³H-methyl]-*S*-adenosylmethionine, radioactivity was incorporated into nonpolar lipid in amounts comparable to that seen with whole cells. However, the heptane-extractable material was not ubiquinone-9. Rather, the nonpolar methylated lipid formed migrated on TLC and eluted on HPLC like fatty acid methyl esters (Fig. 2b). The enzymatic methylation of fatty acids by *S*-adenosylmethionine has been investigated more fully in rat lung membranes [4]. The increased methylation of fatty acids in broken cells is probably due to the activation of phospholipases by homogenation and the consequent greater availability of free fatty acids. Failure to find significant incorporation of radioactivity into ubiquinone in broken cells is consistent with previous results [26, 27]. Biosynthesis of ubiquinone by cell-free systems has been achieved only by careful control of conditions and co-factors [14].

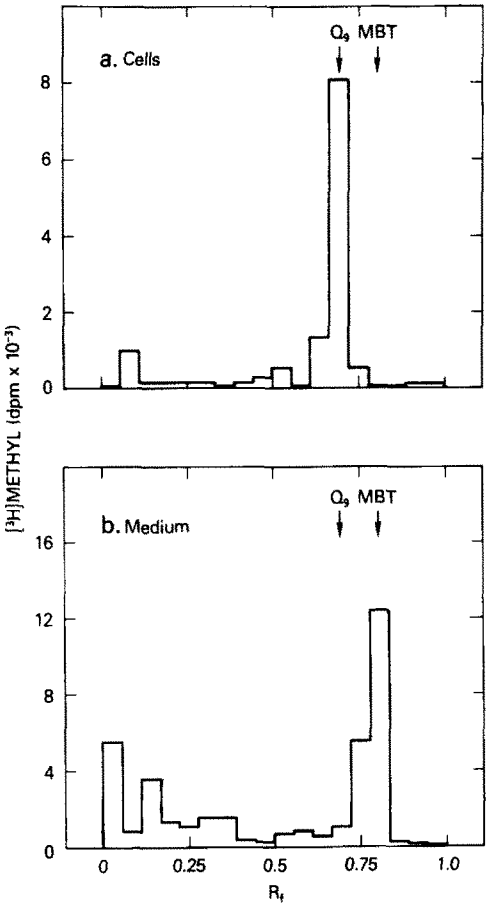

Fig. 5. TLC of methylated nonpolar products in RBL cells and medium. Cells were incubated in Eagle's MEM as described in the legend of Fig. 1. Cells (a) or medium (b) were extracted with alcohol–heptane. The products were separated on TLC in heptane–ether–acetic acid (80 : 30 : 2). The mobilities of 2-(methylthio)-benzothiazole (MBT) and Q_9 standards are indicated.

Table 1. Methylation of lipophilic compounds in retina and parotid preparations

Preparation	[^{3}H]CH_3 incorporated (dpm × 10^{-3}/mg protein)		
	Nonpolar products	Polar products	Non-polar / Polar
Whole rat retina*			
[^{3}H]Methionine	21		
[^{3}H]SAM	24.2		
Minced parotid†			
[^{3}H]Methionine	3.9	20.0	0.19
[^{3}H]SAM	34.7	4.2	8.26
[^{3}H]SAM + 3.5 μM SAH	12.0		

* Two rat retinas were incubated in 5.0 ml of RPMI 1640 medium minus methionine with 50 μCi of [^{3}H-methyl]methionine (15 Ci/mmole) or 50 μCi of [^{3}H-methyl]-*S*-adenosylmethionine (12.9 Ci/mmole) for 60 min at 37°. Retinas were homogenized in 83% ethanol and extracted with heptane.

† Minced rat parotid glands were incubated in 0.5 ml of RPMI 1640 medium minus methionine with 10 μCi of [^{3}H-methyl]methionine or 7.5 μCi of [^{3}H-methyl]-*S*-adenosylmethionine. Reactions were stopped with chloroform–methanol (2 : 1) and extracts were washed three times with 0.1 M KCl–methanol (1 : 1). Polar and nonpolar methylated lipids were separated by TLC using chloroform–methanol–water (65 : 25 : 4) or chloroform respectively. Radioactivity with R_f values between 0.3 and 0.7 in the polar system was taken as a measure of the polar methylated lipids. Radioactivity with R_f values between 0.5 and 0.7 in the nonpolar solvent was taken as a measure of nonpolar methylated products. SAH = *S*-adenosylhomocysteine.

Identification of 2-(methylthio)-benzothiazole. Another nonpolar methylated product was found when RBL cells were incubated in enriched medium. This product migrated with an R_f of 0.7 (similar to fatty acid methyl ester) on TLC using heptane–ether–acetic acid (80 : 30 : 2) (Fig. 5b). However, when the heptane extracts were dried under a stream of nitrogen, more than 75% of the radioactivity was lost. The loss of counts could be prevented by the addition of 0.2 mg/ml of butylated hydroxytoluene. The same semi-volatile product appeared to be formed upon incubation of rat retinas or minced parotid gland in enriched media. Surprisingly, when [^{3}H-methyl]-*S*-adenosylmethionine was used as a precursor, rather than [^{3}H]methionine, comparable or greater amounts of nonpolar methylated product were formed (Table 1). Heptane extracts of parotid incubated with [^{3}H-methyl]-*S*-adenosylmethionine and of retina incubated with [^{3}H-methyl]methionine showed the same major product on HPLC (Fig. 6). HPLC, unlike TLC, resolved this product clearly from fatty acid methyl esters and Q_9 (Figs. 2 and 6).

Methyl-labeled Q_9 was not detected in heptane extracts of retina or parotid incubations. Gas chromatography with radioactive monitoring [4] showed that the methylated product had a retention time similar to that of methyl palmitate on a 3% OV-17 column. However, when a 3% Silar 10 C column was used, it had a retention time similar to that of methyl myristate. The single substance with the required retention characteristics was identified by gas chromatography–mass spectrometry as 2-(methylthio)-benzothiazole (Fig. 7), by computer

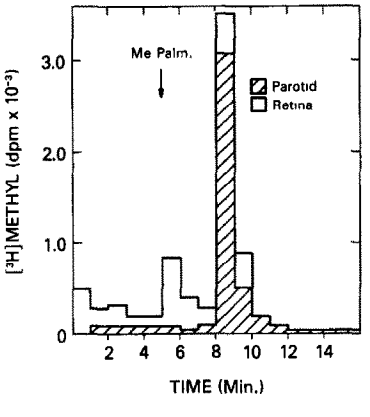

Fig. 6. HPLC of methylated nonpolar product in rat parotid and bovine retina. Parotid minces and whole bovine retina were incubated in RPMI medium with [^{3}H]SAM and [^{3}H]methionine, respectively, as described in Materials and Methods. Nonpolar methylated products were extracted into heptane and separated by using a 15 cm silicic acid (Supelco LC-Si) column eluted with 50% methylene chloride in heptane. The elution profiles from the two experiments have been superimposed. The arrow represents the elution time of [^{14}C]methyl palmitate.

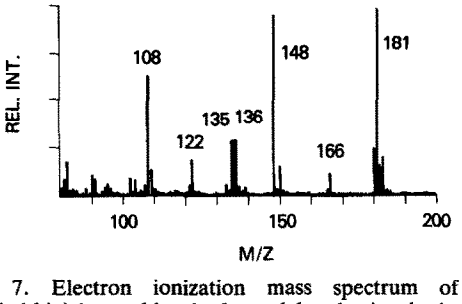

Fig. 7. Electron ionization mass spectrum of 2-(methylthio)-benzothiazole formed by the incubation of bovine retinas in RPMI medium. The HPLC purified extract was analyzed on a 3% OV-17 column at 145°.

comparison of characteristic ions [28]. Subsequent preparation of 2-(methylthio)-benzothiazole from mercaptobenzothiazole by standard methods [29] produced material with HPLC, GC and GC–MS properties identical to those of the substance isolated from retina or parotid incubations. Having identified the product, we looked for the source of the substrate. No thiol substrate was detected in tissue homogenates, tap water, or salt solutions. However, it was found to be present in the enriched media used, at concentrations between 0.01 and 0.1 μM.

An enzyme capable of methylating lipophilic thiols has been described previously. Indeed, 2-thiobenzothiazole was one of the substrates tested [30–33]. The presence of the enzyme was confirmed when we added thiol substrate to parotid homogenates. Addition of 2-thiobenzothiazole increased the yield of heptane-extractable counts more than 20-fold.

"Extracellular" thiomethylation. The ability of the tissues to use exogenous [^{3}H-methyl]-*S*-adenosylmethionine suggested the possibility that thiol-*S*-methyltransferase is present, at least in part, on the surface of the cells. Other data support this conclusion. The addition of exogenous *S*-adenosylhomocysteine (3.5 μM), a competitive inhibitor of methylation by *S*-adenosylmethionine [34], markedly inhibited product formation (Table 1). Neither *S*-adenosylmethionine nor *S*-adenosylhomocysteine penetrates readily into cells. The possibility that the reaction nonetheless reflected uptake of radioactive *S*-adenosylmethionine [35] was examined by comparing the yields of nonpolar product [mostly 2-(methylthio)-benzothiazole] and polar lipid (presumably phospholipid) after incubation with radioactive methionine and *S*-adenosylmethionine (Table 1). The ratio of nonpolar to polar product was less than one to five using [^{3}H]methionine but more than eight to one when [^{3}H]SAM was used. These data suggest that polar lipid was formed preferentially from intracellular *S*-adenosylmethionine (synthesized from methionine) whereas nonpolar product was formed preferentially from extracellular *S*-adenosylmethionine. Finally, if methylation of 2-thiobenzothiazole occurs on the surface of the cells, then both the substrate and the product should be in the medium. The extracellular location of the substrate was described above. Using rat basophilic cells (which can be washed), we found that the methylated thiol formed upon incubation with [^{3}H]methionine in enriched medium was not present in the cells themselves but was present in the medium (Fig. 5). The cells did not make this nonpolar product upon incubation in Tyrode's solution.

Taken together, these results suggest the presence of a thiol-*S*-methyltransferase in retina, parotid, and basophilic leukemia cells, which is, at least in part, able to use extracellular or surface-bound substrates. In view of the suggested role of this enzyme in detoxication [36], this ability is intriguing. The presence of *S*-adenosylmethionine in plasma [37] together with the methyltransferase on the extracellular surface of cells would make it possible to "detoxify" the reactive thiol group before the lipophilic compounds penetrate into the cells.

In the context of endogenous lipid methylation, methylthiobenzothiazole must be considered an interfering substance. It can be avoided by (1) incubating cells in balanced salt solution rather than enriched medium, (2) washing the cells, and (3) thorough drying of extracts. Adequacy of avoidance or removal can be checked by HPLC, which readily distinguishes methylthiobenzothiazole from the nonpolar methylated lipids formed endogenously by whole cells and homogenates.

Using these approaches we have demonstrated that ubiquinone-9 is the major methylated product made by whole RBL cells, but not by retina or minced parotid. Significant formation of this product may require growing cells. Fatty acid methyl esters, on the other hand, were the major nonpolar methylated lipids formed by homogenates of cells like RBL, retina, parotid (unpublished observations) and lung [4]. They are also formed by whole cells. Nonpolar lipids constitute a significant portion of the methylated lipid products formed upon incubation of cells, tissues, and homogenates with radioactive methionine or *S*-adenosylmethionine. They may contribute to some of the effects of methylation on cellular processes.

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REFERENCES

1. E. Usdin, R. T. Borchardt and C. R. Creveling, *Transmethylation*. Elsevier/North Holland, Amsterdam (1978).
2. F. Hirata and J. Axelrod, *Science* **209**, 1082 (1980).
3. F. T. Crews, Y. Morita, F. Hirata, J. Axelrod and R. P. Siraganian, *Biochem. biophys. Res. Commun.* **93**, 42 (1980).
4. M. Zatz, P. A. Dudley, Y. Kloog and S. P. Markey, *J. biol. Chem.* **256**, 10028 (1981).
5. H. M. Fales, T. M. Jaouni and J. F. Babashak, *Analyt. Chem.* **45**, 2302 (1973).
6. A. Kulczycki, Jr. and H. Metzger, *J. exp. Med.* **140**, 1676 (1974).
7. J. D. Taurog, G. R. Mendoza, W. A. Hook, R. P. Siraganian and H. Metzger, *J. Immun.* **119**, 1757 (1977).
8. E. L. Barsumian, C. Isersky, M. G. Petrino and R. P. Siraganian, *Eur. J. Immun.* **11**, 317 (1981).
9. D. C. Morrison, J. F. Roser, P. M. Henson and C. G. Cochrane, *J. Immun.* **112**, 573 (1974).
10. P. K. Chaing, H. H. Richards and G. L. Cantoni, *Molec. Pharmac.* **13**, 939 (1977).
11. H. Elliott and G. R. Walker, in *Vitamins and Cofactors in Biomedical Applications of Mass Spectrometry* (Ed. G. R. Waller), p. 521. Wiley Interscience, New York (1972).
12. T. Nakamura, I. Y. Sawa, J. Hasegowa, K. Shaguchi, T. Fujita, Y. Mori and K. Kawabe, *Analyt. Chem.* **51**, 534 (1979).
13. D. E. M. Lawson and J. Glover, *Biochem. biophys Res. Commun.* **4**, 223 (1961).
14. B. R. Trumpower, R. M. Houser and R. E. Olson, *J. biol. Chem.* **249**, 3041 (1974).
15. F. L. Crane and R. Barr, in *Methods in Enzymology* (Eds. D. B. McCormick and L. D. Wright), Vol. XVIII, p. 220. Academic Press, New York (1971).

16. J. Lars, G. Nilsson, T. M. Farley and K. Folkers, *Analyt. Biochem.* **23**, 422 (1968).
17. B. O. Linn, N. R. Treener, B. H. Arison, R. G. Weston, C. H. Shunk and K. Folkers, *J. Am. chem. Soc.* **82**, 1967 (1960).
18. J. Jayaraman and T. Ramasarama, *Archs Biochem. Biophys.* **103**, 258 (1963).
19. H. Rudney and T. Sugimura, in *Quinones in Electron Transport* (Eds. G. E. W. Wolstenholme and C. M. O'Connor), p. 211. Churchill, London (1961).
20. A. M. D. Nambudiri, S. Ranganathan and H. Rudney, *J. biol. Chem.* **255**, 5897 (1980).
21. J. R. Faust, J. L. Goldstein and M. S. Brown, *Archs Biochem. Biophys.* **192**, 86 (1979).
22. R. A. Leppik, P. Stroobant, B. Shineberg, I. G. Young and F. Gibson, *Biochim. biophys. Acta* **428**, 146 (1976).
23. A. M. D. Nambudiri, D. Brockman, S. S. Alam and H. Rudney, *Biochem. biophys. Res. Commun.* **76**, 282 (1977).
24. T. Ramasarma, *Adv. Lipid Res.* **6**, 107 (1968).
25. M. Klingenberg, in *Biological Oxidations* (Ed. T. P. Singer), p. 3. Wiley Interscience, New York (1968).
26. S. Ranganathan, A. M. D. Nambudiri and H. Rudney, *Archs Biochem. Biophys.* **198**, 506 (1979).
27. R. E. Olson, in *Proceedings of the Fifth International Congress of Biochemistry* (Ed. G. Popjak), Vol. 7, p. 322. Pergamon Press, New York (1961).
28. Mass Spectral Search System, NIH-EPA Chemical Information System, Washington, DC.
29. K. Blau and G. King, *Handbook on Derivatives for Chromatography*. Heyden, London (1977).
30. R. A. Weisiger and W. B. Jackoby, *Archs Biochem. Biophys.* **196**, 631 (1979).
31. J. Bremer and D. M. Greenberg, *Biochim. biophys. Acta* **46**, 217 (1961).
32. D. T. Borchardt and C. F. Cheng, *Biochim. biophys. Acta* **522**, 340 (1978).
33. T. Fujita and Z. Suzuoki, *J. Biochem., Tokyo* **74**, 717 (1973).
34. J. Axelrod, in *Concepts in Biochemical Pharmacology* (Eds. B. B. Brodie and J. R. Gillette), Vol. 2, p. 609. Springer, Berlin (1971).
35. G. Stramentinoli and C. Pezzoli, in *Transmethylation* (Eds. E. Usin, R. T. Borchardt and C. R. Creveling), p. 37. Elsevier/North Holland, Amsterdam (1978).
36. R. A. Weisiger, L. M. Pinkus and W. B. Jakoby, *Biochem. Pharmac.* **29**, 2885 (1980).
37. W. K. Paik and S. Kim, *Protein Methylation,* p. 21. John Wiley, New York (1980).