sedimentation coefficients $\left(\bar{s} = \sum_{i=0}^{n} \alpha_i s_i \text{ where } \alpha_i \text{ is the weight fraction appearing as the } i^{th} \text{ species}\right)$. Different methods can be used to evaluate $\bar{s}_A$.[5] The term $(\bar{s}_P - s_P)$ which may be very small, about 0.05 svedbergs, can be measured accurately by the differential technique. The same optical principles may be useful in the direct measurement of small changes in molecular weights and diffusion coefficients.

(6) du Pont Fellow in Biochemistry. This work is submitted in partial fulfillment of the requirements for the Ph.D. degree in Biochemistry at the University of California.

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THE ENZYMATIC SYNTHESIS OF SPHINGOMYELIN[1]

Sir:

It has been suggested[2] that the enzymatic synthesis of sphingomyelin may take place by the transfer of the phosphorylcholine moiety of cytidine diphosphate choline (CDP-choline) to the primary hydroxyl group of an N-acylsphingosine (ceramide) in a reaction fundamentally similar to the enzymatic synthesis of lecithin[3]

$$\text{Cyt-P-P-choline} + \text{Ceramide} \rightleftarrows \text{Sphingomyelin} + \text{Cyt-P} \quad (1)$$

An enzyme (phosphorylcholine-ceramide transferase) has now been found in chicken liver which catalyzes an extensive net synthesis of sphingomyelin from C^{14}-labeled CDP-choline and an "active ceramide." When the enzyme incubation was run on a large scale (100 ml.) under the conditions described in Table I, 28.5 mg. of enzymatically synthesized phospholipide was isolated by chromatography on silicic acid and identified as sphingomyelin by the following properties: stability toward mild alkaline hydrolysis; insolubility in ether and acetone; N/P ratio of 2.08; and an infrared spectrum closely resembling that of purified sphingomyelin. The purity of the isolated product, based on comparison of its specific radioactivity with that of the CDP-choline used, was at least 90%.

The enzyme is highly specific, both for CDP-choline and "active ceramide." Ceramides with short-chain fatty acids in amide linkage are much more active than long-chain fatty acid amides, presumably because the short chain compounds are more soluble and penetrate more readily to the enzyme surface. N-Acetyldihydrosphingosine and N-acetylphytosphingosine are inactive (Table I). Acetylation of *crude* sphingosine (obtained by acid hydrolysis of cerebrosides) with acetic anhydride in the presence of alkali[4] leads to the formation of "active ceramide." In contrast, N-acetylsphingosine prepared by treatment of recrystallized triacetylsphingosine with alkali shows little activity, but it is converted to "active ceramide" by heating with acetic–sulfuric acid. It is concluded that the sphingosine portion of "active ceramide" does not possess the D-erythro-*trans*-sphingosine structure which has been definitely established for the triacetylsphingosine described by Carter[4,5] and the N-acetylsphingosine derived from it. This result is unexpected in view of the evidence[5] that naturally occurring sphingolipides are also of the D-erythro-*trans* structure.

Two possibilities appeared most likely. The "active ceramide" might either have the *cis* rather than the *trans* configuration at the double bond, or the *threo* rather than the *erythro* relationship of the amino group to the hydroxyl on C-3. Generous

TABLE I

REQUIREMENT FOR "ACTIVE CERAMIDE" IN SPHINGOMYELIN SYNTHESIS

	Additions	Sphingomyelin synthesized, milli-micromoles
1	Sphingosine	2
2	N-Acetyldihydrosphingosine	1
3	N-Acetylphytosphingosine[6]	1
4	"Active ceramide" (mixture of N-acetyl-sphingosine isomers)	85
5	N-Acetylsphingosine derived from recrystallized triacetylsphingosine[4]	17
6	N-Acetylsphingosine after treatment with acetic–sulfuric acid	155

Each tube contained 50 μmoles of Tris buffer, *p*H 7.4, 20 μmoles of cysteine, 4 μmoles of $MnCl_2$, 0.8 μmole of CDP-choline labeled with choline-1,2-C^{14}, 5 mg. of Tween-20 (polyoxyethylene sorbitan monolaurate), and 0.25 ml. of a suspension of particles obtained from 0.25 *M* sucrose homogenates of chicken liver by a method already described.[3] Four μmoles of substances to be tested were added as indicated. The final volume of the system was 1.0 ml., and the incubation was for 2 hours at 37°, after which the lipides were extracted repeatedly with hot methanol. The lipide extract was hydrolyzed in 0.4 *N* methanolic potassium hydroxide at 37° for two hours, transferred to chloroform and thoroughly washed.[3] Aliquots of the chloroform solution were then plated and counted.

TABLE II

ACTIVITY OF ISOMERS OF N-ACETYLSPHINGOSINE AS ENZYMATIC PRECURSORS OF SPHINGOMYELIN

Additions	Sphingomyelin synthesized millimicromoles
Experiment 1	
N-Acetyl-DL-erythro-*trans*-sphingosine[6]	4
N-Acetyl-DL-threo-*trans*-sphingosine[6]	105
Experiment 2	
N-Acetyl-DL-erythro-*trans*-sphingosine[7]	5
N-Acetyl-DL-threo-*trans*-sphingosine[7]	83
N-Acetyl-DL-erythro-*cis*-sphingosine[7]	1
N-Acetyl-DL-threo-*cis*-sphingosine[7]	1

The conditions of the experiment were the same as shown in Table I. Four μmoles of the N-acetyl derivatives of the sphingosine isomers was added as shown.

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gifts[6,7] of synthetic isomers of sphingosine made it possible to settle this point in the experiments shown in Table II in which "active ceramide" is identified as N-acetyl-DL-threo-*trans*-sphingosine.

The possible biological significance of the fact that the enzymatically active form of sphingosine possesses the *threo* rather than the expected *erythro* configuration is currently being investigated.

(6) Gift of Dr. Paul O'Connell and the Upjohn Company.
(7) Gift of Prof. C. A. Grob and the Ciba Company.

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THE REACTION OF THIYL RADICALS WITH TRIALKYL PHOSPHITES[1]

Sir:

Hoffmann and co-workers[2] recently have reported a remarkable reaction between mercaptans and trialkyl phosphites occurring at elevated tempera-

$$RSH + P(OEt)_3 \longrightarrow RH + SP(OEt)_3 \quad (1)$$

tures, or photochemically at room temperature. They make no suggestion as to reaction mechanism, but the light catalysis suggests a radical process. We find that the reaction is initiated by other free radical sources and evidently involves long radical chains. Thus, isobutyl mercaptan and a slight excess of triethyl phosphite show negligible reaction in 30 min. at 70°. In the presence of 2 mole % azobisisobutyronitrile reaction is complete in two minutes, from which we calculate a minimum chain length (from the known decomposition rate of the initiator) of 2700.

A quite analogous radical reaction between disulfides and trialkyl phosphites may be induced photochemically at 60° or by di-*t*-butyl peroxide at 120–125°, and follows the course

$$RSSR + P(OEt)_3 \longrightarrow RSR + SP(OEt)_3 \quad (2)$$

Equivalent quantities of isobutyl disulfide and triethyl phosphite react almost quantitatively in 3 hours at 60° on irradiation with a General Electric RS Sunlamp, the reaction being followed conveniently by gas chromatographic analysis of aliquots. The phosphorothionate was identified by fractional distillation and comparison of its infrared spectrum with an authentic sample. Similarly, equimolecular quantities of the same components show 60–70% reaction in 3 hours at 120–125° in the presence of 2 mole % of di-*t*-butyl peroxide, indicating a minimum kinetic chain of 400.[3] Negligible reaction occurs at 140° in 3 hr. in the absence of peroxide, and azobisisobutyronitrile is ineffective as an initiator at 60–100°. This contrast with the mercaptan reaction suggests that chain initiation in both cases involves initiator radical attack upon the sulfur compound.

Jacobson, Harvey and Jensen[4] have described the reaction of diethyl disulfide and triethyl phosphite on refluxing at a high temperature to give the phosphorothiolate, $OP(SEt)(OEt)_2$, which they consider to be an Arbuzov-type polar process. While our results do not rule out this alternative reaction, their reaction may well follow our path, since phosphorothionates readily undergo thermal isomerization to phosphorothiolates.[5] We find that the original gas chromatographic peak of triethyl phosphorothionate almost disappears on heating for 23 hr. at 180°.

We propose these chain processes for the reactions, involving thiyl radical attack on phosphorus to yield a phosphorus radical with an expanded valence shell

$$RS\cdot + P(OEt)_3 \longrightarrow RS\text{–}\dot{P}(OEt)_3 \quad (3)$$

$$RS\text{–}\dot{P}(OEt)_3 \longrightarrow R\cdot + SP(OEt)_3 \quad (4)$$

$$R\cdot + HSR \longrightarrow RH + RS\cdot \quad (5a)$$

$$R\cdot + RSSR \longrightarrow RSR + RS\cdot \quad (5b)$$

(5a) and (5b) representing the usual chain transfer reactions of mercaptans and disulfides respectively. Certain limitations of the reaction are compatible with this formulation. Thus, judged by chain transfer experiments with styrene,[6] benzyl-type radicals react only sluggishly with disulfides. Dibenzyl disulfide (0.04 mole) shows little reaction in 14 hr. on irradiation with triethyl phosphite (0.2 mole). In three weeks, at least 63% of the sulfur appears as crude phosphorothionate, while the benzyl radicals are converted to 19% toluene and 26% bibenzyl. Here there is evidently little chain reaction, but the latter products arise from benzyl radicals formed in (4).

It also is of interest that similar reaction intermediates involving an expanded valence shell of phosphorus appear to account for the products obtained in the reaction of *t*-phosphines with polyhalomethanes.[7]

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(8) Columbia University Fellow, 1956–1957.